SOME EFFECTS OF THE LOWER ALCOHOLS ON *PARAMECIUM*.[1]

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General—Investigators of the protozoa do not agree as to the influence of abnormal environment. For example, Matheny (1910) states that alcohol in doses of two per cent. or less "has no effect whatever" on *Paramecium,* while Calkins and Lieb (1902), and Woodruff (1908), working with doses many times more dilute report marked, but dissimilar effects.

In the present studies considerable variation in the deportment of individual paramecia from a given clone was noted, which indicates that some of the factors of error in the quantitative study of *Paramecium* are obscure, and not easy of control. As Towle (1904) observes, "The sensitiveness of paramecia for different substances varies without apparent regularity." Nevertheless it was found possible in the following experiments to obtain results of significance by counting great numbers of organisms, observing strict chemical cleanliness, and confining most of the experiments to dormant cultures of pure stocks.

Cultures—Pure lines of *Paramecium caudatum* and *Paramecium aurelia* were cultivated in battery jar infusions consisting of about 25 grams of timothy hay per liter of spring water. These were twice boiled to insure the destruction of rotifers. After about a month from the date of preparation the cultures entered upon a prolonged stage of dormancy during which little detectable change occurred until starvation was evidenced by an abrupt decline. Except where otherwise noted, only organisms from the dormant cultures were studied.

No attempt was made at bacterial control. However, in one culture a mixture of *B. lactis aerogenes* and a bacillus of the

[1] Abbreviated excerpts from an essay presented to The Johns Hopkins University in conformity with the requirements for the degree of Master of Arts (Bills, 1923*a*). A previous publication (Bills, 1923*b*) containing other excerpts should be consulted.

aquatilis group gained a long-enduring ascendency over all other bacterial forms.[2] As this culture supported the finest growth of paramecia that I have ever seen, it is interesting to note that Hargitt and Fray (1917) and Phillips (1922) maintain that simple bacterial mixtures do *not* provide as good a food for *Paramecium* as the usual complex natural mixtures.

Alcohols—The six simplest monatomic alcohols were employed: Methyl, ethyl, *n*-propyl, *i*-propyl, *n*-butyl, and *i*-butyl. All were of good purity. Dilutions in spring water were prepared volumetrically, with a micro-burette, a fresh solution being employed for each observation.

The Effect of Lethal Concentrations of the Alcohols—One clone of *P. aurelia* and two of *P. caudatum* were treated with such strengths of the four normal alcohols as sufficed to kill them in from 30 seconds to 30 minutes. The strongest concentration used was 15 per cent methyl alcohol; the weakest was 0.8 per cent butyl. All other strengths were intermediate. In about 300 individuals the process of dying was observed under a magnification of 700 diameters.

Wide variations disregarded, the phenomena usually observed follow in order: Incoördination and inactivation of body cilia; discharge of trichocysts; arrest of contractile vacuoles; modification of cyclosis in course and diminution in rate; bending of body to a crescent; convulsive rearrangement in posterior part, producing "Indian club" shape; arrest of undulating membrane in gullet; and at death, change in appearance of protoplasm to opaque and yellowish, cessation of Brownian movement, occasional formation of blisters by elevation of cuticle, and sometimes rupture of ectoplasm with discharge of endoplasm into the blisters.

Three points in blister formation warrant further mention: (1) The lower alcohols give rise to a *few* blisters which grow *rapidly,* whereas the higher ones result in *many* blisters which grow *slowly.*

(2) The existence of "susceptibility gradients" in *Paramecium* is nicely demonstrated in blister formation. Blisters

[2] I am much indebted to Dr. Percy D. Meader, of the School of Hygiene and Public Health, for the bacteriological examination of this unusual culture.

rarely, if ever, form in the oral groove, and this region is generally the last to exhibit the signs of death. But the anterior end is the most susceptible to blister formation, and it is there that the ectoplasm most frequently breaks. Furthermore, the aboral side is more susceptible than the oral, being nearly as delicate as the anterior end, in regard to both blister formation and ectoplasmic rupture. In this connection it is interesting to recall the work of Child (1914) who demonstrated in *Paramecium* anterior hypersensitiveness to cyanide.

(3) When the granules of the seemingly still living protoplasm are discharged thru the ectoplasm into a blister they do not behave precisely like free particles in a liquid; they keep together in globular masses, or in thread-like protrusions. Sometimes they may become differentiated even more distinctly from the still hyaline portion of the blister by forming a new superficial film. These observations are in accord with the researches of Seifriz (1921), who noted the tendency of living protoplasm to remain immiscible with water, and "to form, almost instantly, a membrane on its surface."

Alcohol and Resistance to Starvation—None of the earlier studies on the influence of alcohol on *Paramecium* appears to have considered the effect on starving cultures. In attacking this problem cultures were prepared by adding one volume of dormant stock culture to one volume of an alcohol of twice the desired strength. Such mixtures were apportioned in 25 cc. fractions to about 100 Stender dishes of 30 cc. capacity. Most of the dishes were kept at room temperature, and the covers removed only when observations were made at various intervals. A few of the cultures were temperature-controlled.

By a method described at length in my original essay (Bills, 1923*a*) determinations were made on the maintenance of the alcoholic content of these cultures. It was found that in spite of the closely fitting covers on the dishes the alcoholic content diminished at the rate of 21 per cent. of the original amount in five days, and 56 per cent. in 31 days, these values including loss by consumption as well as loss by evaporation. Both values are averages of 45 cultures containing 1.25 per cent. ethyl alcohol.

Three sets of observations were made. The results are recorded in Tables I., II., and III. Table I. is a record of the ac-

TABLE I.

A RECORD OF THE ACTIVITY, SIZE, AND POPULATION OF CULTURES FROM CLONE 10, UNDER THE INFLUENCE OF 1.0 PER CENT. METHYL, 0.8 PER CENT. ETHYL, 0.4 PER CENT. PROPYL, AND 0.2 PER CENT. BUTYL ALCOHOLS.

Alcohol.	Exposure.	Activity.	Size.	Population.
Methyl ..	24 Hours	Normal	Decreased	
Ethyl....		Increased	Normal	
Propyl...		Normal	Normal	
Butyl....		Increased	Normal	
Methyl ..	64 Hours	Normal	Normal	Distributed throughout culture
Ethyl....		Normal	Normal	Distributed throughout culture
Propyl...		Normal	Normal	Aggregated in dense masses
Butyl....		Normal	Normal	Distributed throughout culture
Methyl ..	91 Hours	Decreased	Decreased	
Ethyl....		Normal	Normal	
Propyl...		Decreased	Decreased	
Butyl....		Normal	Normal	
Methyl ..	25 Days		Decreased	Very few
Ethyl....			Normal	Few
Propyl...			Normal	Extremely numerous
Butyl....			Normal	Extremely numerous
Methyl ..	53 Days	Decreased		Few
Ethyl....		Decreased		Very numerous
Propyl...		Decreased		Numerous
Butyl....		Decreased		Numerous
Methyl ..	120 Days			Extinct
Ethyl....				Very few
Propyl...				Extinct
Butyl....				Extinct

tivity, size, and endurance of organisms from *Paramecium caudatum*, Clone 10 exposed for varying lengths of time to the first four normal primary alcohols in the following concentrations: 1.0 per cent. methyl; 0.8 per cent. ethyl; 0.4 per cent. propyl; 0.2 per cent. butyl. Table II. is a similar record of five discrete experiments on Clone 10 showing the difference between alchoholized and normal cultures. Controls consisting of culture diluted with an equal volume of spring water were used in this series. All the treated cultures contained 1.0 per cent. methyl alcohol. Table III. is a comprehensive record of seven cultures of paramecia,

each of which was treated with six different alcohols, and observed as to population in comparison with untreated, undiluted, controls at intervals up to two months from the time of preparation. The concentrations of methyl and *n*-propyl alcohols seem to have been a little high for some of the cultures. The alcoholic content was as follows: 2 per cent. methyl; 1 per cent. ethyl; 1/2 per cent. *n*-propyl; 1/4 per cent. *n*-butyl; 3/4 per cent. *i*-propyl; 3/8 per cent. *i*-butyl.

TABLE II.

RECORDS OF FIVE DISCRETE EXPERIMENTS ON CLONE 10 IN 1.0 PER CENT. METHYL ALCOHOL, SHOWING THE DIFFERENCES BETWEEN ALCOHOLIZED AND CONTROL CULTURES AFTER DIFFERENT PERIODS OF TIME.

1. Time, 16 days. Temperature maintained at 27.5°.
   Treated culture flourishing.
   Control beginning to starve.
2. Time, 30 days. Room temperature. Covers sealed with vaseline.
   Treated culture contains many large, slow-moving animals.
   Control died of starvation.
3. Time, 30 days. Temperature maintained at 25°.
   Treated culture contains many paramecia of almost normal size, but much vacuolated and very slow-moving.
   Control died of starvation.
4. Time, 30 days. Temperature maintained at 35°.
   Treated culture contains many small, active paramecia.
   Control died of starvation.
5. Time, 50 days. Room temperature.
   Treated culture contains many large, active, slightly vacuolated paramecia.
   Control died of starvation.

Inspection of the tables reveals that all alcohols have a similar influence on starving cultures. Not only do all of them postpone the advent of starvation, but they may even restore severely starved cultures to their former prosperity. This fact should not be taken to indicate that alcohols function *directly* as food for *Paramecium*, as they appear to do for green algæ (Moore and Webster, 1920). In the present case their mode of action is obscure. In activity the alcoholized paramecia remain normal, increase, or decrease; and in size they remain normal, or decrease—conditions attributable quite as well to nutritional as to pharmacological influence.

TABLE III.

A POPULATION RECORD OF SEVEN CULTURES EXPOSED, WITH CONTROLS, TO SIX ALCOHOLS FOR DIFFERENT PERIODS OF TIME.

The alcoholic strengths were: 2 per cent. methyl; 1 per cent. ethyl; ½ per cent. *n*-propyl; ¼ per cent. *n*-butyl; ¾ per cent. *i*-propyl; ⅜ per cent. *i*-butyl.

	Alcohol.	Population at Time of Prep.	Population 4 Days Later.	Population 30 Days Later.	Population 60 Days Later.
P. caudatum Clone 10	Control	Pale, odorless, nearly extinct from starvation.	Nearly extinct	Nearly extinct	Extinct
	Methyl		Extinct	.....	.....
	Ethyl		Multiplying	Very numerous	Excellent
	n-Propyl		Extinct	.....	.....
	n-Butyl		Slight increase	Very numerous	Numerous thin
	i-Propyl		Nearly extinct	Numerous	Extinct
	i-Butyl		Extinct	.....	.....
P. caudatum Clone 10	Control	Pale, putrid, extremely numerous. Some aggregates of paramecia have been broken up.	Excellent	Very numerous	Very few thin
	Methyl		Excellent thin	Numerous fat	Extinct
	Ethyl		Excellent	Excellent	Numerous small
	n-Propyl		Extinct	.....	.....
	n-Butyl		Excellent	Very numerous	Numerous
	i-Propyl		Numerous	Numerous	Extinct
	i-Butyl		Excellent	Numerous	Few but good
P. caudatum Clone 8	Control	Pale, odorless, dormant. Will soon be starving.	Excellent	Very few	Extinct
	Methyl		Nearly extinct	Extinct	.....
	Ethyl		Excellent	Very few	Very few
	n-Propyl		Extinct	.....	.....
	n-Butyl		Excellent	Few	Extinct
	i-Propyl		Excellent	Few	Extinct
	i-Butyl		Excellent	Very numerous	Nearly extinct
P. aurelia Clone 11	Control	Colorless, odorless, few paramecia in starvation.	Excellent	Few	Extinct
	Methyl		Few	Very few	Numerous good
	Ethyl		Excellent	Nearly extinct	Extinct
	n-Propyl		Extinct	.....	.....
	n-Butyl		Numerous	Nearly extinct	Extinct
	i-Propyl		Excellent	Extinct	.....
	i-Butyl		Very few	Extinct	.....
P. aurelia Clone 7	Control	Pale, odorless, dormant.	Numerous	Nearly extinct	Extinct
	Methyl		Few	Extinct	.....
	Ethyl		Numerous	Numerous	Accident
	n-Propyl		Extinct	.....	.....
	n-Butyl		Multiplying	Very numerous	Very few
	i-Propyl		Numerous	Few	Extinct
	i-Butyl		Numerous	Extinct	.....
P. aurelia Clone 4	Control	Pale, odorless. Few paramecia, somewhat starved.	Numerous	Very numerous	Few thin
	Methyl		Numerous	Very numerous	Few good
	Ethyl		Multiplying	Nearly extinct	Few thin
	n-Propyl		Extinct	.....	.....
	n-Butyl		Numerous	Nearly extinct	Numerous
	i-Propyl		Numerous	Numerous	Accident
	i-Butyl		Very few	Numerous	Numerous
P. caudatum Wild culture	Control	Colorless, putrid. The aggregates of paramecia have been well broken up. Extremely numerous.	Extinct	.....	.....
	Methyl		Extinct	.....	.....
	Ethyl		Few	Excellent	Numerous thin
	n-Propyl		Few	Very numerous	Numerous small
	n-Butyl		Very numerous	Few thin	Nearly extinct
	i-Propyl		Few	Very few	Few thin
	i-Butyl		Numerous	Excellent	Numerous thin

The Influence of Temperature on the Susceptibility of Paramecium to Ethyl Alcohol.—Within reasonable limits of constancy a given concentration of a given alcohol will narcotize a definite per cent. of the organisms in a particular culture in one hour. This fact makes it possible to compare quantitatively the narcotic action of various alcohols, and to study the modifying influence of physical conditions on the effects of a particular alcohol. The method devised for counting the paramecia "narcotized" and those "unaffected" is elsewhere described (Bills, 1923*b*).

In the present experiment counts were made at widely different temperatures—8° and 25°. *Paramecium caudatum,* Clone 10, and 3.0 per cent. ethyl alcohol were used, and all observations made in duplicate. From the data presented in Table IV. it appears that the per cent. of the paramecia narcotized at 8° does not differ significantly from the per cent. at 25°. It seems improbable (though of course possible) that intermediate temperatures would show any markedly different values.

TABLE IV.

SHOWING THE INFLUENCE OF TEMPERATURE ON THE SUSCEPTIBILITY OF PARAMECIA TO ETHYL ALCOHOL.

The experiments were conducted in darkness.

Temperature.	Number of Paramecia Narcotized.	Number of Paramecia Unaffected.	Per Cent. of Paramecia Narcotized.
8°	50	793	5.9
8°	43	473	8.3
25°	47	568	7.6
25°	32	358	8.2

Average per cent. narcotized at 8° = 7.1.
Average per cent. narcotized at 25° = 7.9.

The Influence of Light on the Susceptibility of Paramecium to Ethyl Alcohol.—Pairs of burettes containing paramecia of Clone 10, with and without 3.0 per cent. ethyl alcohol were kept for one hour in strong, but diffuse, northern, daylight, or in direct, brilliant, sunlight in the middle of April. The direct light passed obliquely through the thin glass walls of the burettes. In all experiments the temperature was between 21° and 23°.

The data are presented in Table V. This table shows that direct sunlight inactivated in one hour 28 per cent. of the organ-

isms in plain spring water, but that under like conditions, except that 3.0 per cent. ethyl alcohol was present, the sunlight inactivated 42 per cent. The experiments were repeated in diffuse daylight, and not one individual was inactivated in the absence of alcohol, while with 3.0 per cent. alcohol 8.9 per cent. were affected—a figure not significantly different from the values got in the temperature experiments which were made in darkness.

TABLE V.

SHOWING THE INFLUENCE OF LIGHT ON THE SUSCEPTIBILITY OF PARAMECIA TO ETHYL ALCOHOL.

Light.	Per Cent. Alcohol.	Number of Paramecia Narcotized.	Number of Paramecia Unaffected.	Per Cent. of Paramecia Narcotized.	Average.
Diffuse daylight....	0.0	0	1361	0.0	0.0
Diffuse daylight....	3.0	213	1641	12	
Diffuse daylight....	3.0	28	454	5.8	8.9
Direct sunlight.....	0.0	558	1432	28	
Direct sunlight.....	0.0	451	1150	28	28
Direct sunlight.....	3.0	888	914	49	
Direct sunlight.....	3.0	726	1306	35	42

The Combined Effect of Preliminary Aëration and Agitation of a Paramecium Culture on its Subsequent Susceptibility to an Alcohol.—Aëration was effected by agitating for two minutes some paramecia of Clone 10 in 1.6 per cent. of *i*-propyl alcohol. This alcohol was chosen because of the fine froth produced when cultures containing it are violently shaken. The results presented in Table VI. show that the aërated paramecia are decidedly less susceptible than normal controls. Of the agitated organisms 17 per cent. were narcotized, whereas 32 per cent. were narcotized in the non-aërated control culture.

TABLE VI.

SHOWING THE INFLUENCE OF AËRATION AND AGITATION ON THE SUSCEPTIBILITY OF PARAMECIA TO 1.6 PER CENT. *i*-PROPYL ALCOHOL.

Treatment of Culture.	Number of Paramecia Narcotized.	Number of Paramecia Unaffected.	Per Cent. of Paramecia Narcotized.
Agitated and aërated....	239	1139	17
Normal control.....	682	1460	32

The Question of the Adaptation of Parmecium to Alcohol.— How does a preliminary exposure of *Paramecium* to a low concentration of ethyl alcohol affect the subsequent resistance to a stronger dose of ethyl alcohol, and of its homologues?

Towle (1904), working with electrolytes and simple organic compounds, concluded that "paramecia become readily habituated to solutions in strengths which are not soon fatal." Daniel (1908) found that paramecia when transferred *gradually* into distilled water become adjusted to this otherwise deadly substance. Estabrook (1910) developed in *Paramecium* a temporarily increased tolerance for strong doses of sodium chloride. Neuschlosz (1921*a*) found that *Paramecium* can develop a high resistance to dyes of the thiazin, benzidin, and triphenylmethane series. Neuschlosz later (1921*b*) reported that paramecia acclimatized to trivalent arsenic are at the same time resistant to trivalent antimony. Woodruff (1908) observed that alcoholized paramecia become more sensitive to copper sulphate. Their behavior toward a stronger dose of *alcohol* was not recorded. A case of adaptation in *Spirostomum* and *Stentor* reported by Daniel (1909) is of special interest, inasmuch as the method of experimentation is essentially identical with my method on *Paramecium;* the results, however, being different from mine. Daniel claims that he sometimes produced in these protozoa a slight adaptation to ethyl alcohol, but that this was accompanied by an increased susceptibility to methyl alcohol.

My experiments were made as follows: To 10 cc. of the Clone 10 culture taken from near the surface 10 cc. of 2.0 per cent. ethyl alcohol was added, making a 1.0 per cent. solution of alcohol. This mixture was put into a 30 cc. Stender dish and kept at approximately 24° for three days. At the end of this period the paramecia were observed to be distributed thruout the medium, appearing healthy, and distinctly more active than the controls altho possibly somewhat thinner. They were then exposed for one hour to each of the six alcohols in the concentrations indicated in Table VII., using quantities large enough to eliminate practically all error resulting from the presence of the original ethyl alcohol (see Bills, 1923*a*).

The results obtained are presented in Table VII. In this

table the values given for the untreated controls are interpolated averages obtained for the six alcohols in a series of experiments elsewhere described (Bills, 1923*b*). They were made a few days before the present experiment was performed, during a period of extended constancy in the cultures. Therefore, these values are admissible for comparison here, and are probably more nearly accurate than single observations would have been.

From Table VII. it is clear that the three-day exposure to 1.0 per cent. ethyl alcohol increased the susceptibility of the paramecia to a narcotizing concentration of ethyl alcohol; and, similarly, to each of the other five alcohols. In other words, paramecia are not acclimatized to ethyl alcohol under the conditions of this experiment. Unlike Daniel's spirostoma and stentors which under similar conditions became more resistant to ethyl alcohol, paramecia became more susceptible to *all* alcohols.

TABLE VII.

SHOWING THE EFFECT OF EXPOSURE TO ALCOHOL ON THE ACTION OF ALCOHOLS ON *Paramecium*.

Period of Acclimatization to 1.0 Per Cent. Ethyl Alcohol:	Narcotizing Alcohols.	Per Cent. of Treated Paramecia Narcotized.	Per Cent. of Untreated Paramecia Narcotized.
72 hours.....	Methyl, 5.0%	Mostly disintegrated	30
72 hours.....	Ethyl, 3.3%	Many disintegrated	24
77 hours.....	*n*-Propyl, 0.9%	35	31
77 hours.....	*n*-Butyl, 0.5%	44	31
79 hours.....	*i*-Propyl, 1.6%	55	29
79 hours.....	*i*-Butyl, 0.4%	54	35

I wish to express my appreciation of the interest and guidance given me in the course of these experiments by Professor S. O. Mast and Professor H. S. Jennings; and my thanks to many other persons for their assistance in many ways.

SUMMARY.

1. A mixture of *B. lactis aerogenes* and *B. aquatilis* (sp. ?) constitutes the best food found for *Paramecium*.

2. When paramecia are exposed to an alcohol in sufficient strength they are at first incoördinated in movement and then inactivated. Later toxic effects are manifested by marked in-

ternal changes, formation of "blisters" by elevation of cuticle, rupture of ectoplasm, and death.

3. The anterior end of *Paramecium* is more susceptible to alcohol than the posterior end, and the aboral side more than the oral.

4. Indirect daylight has no perceptible effect on normal or alcoholized paramecia, but direct sunlight inactivates them; this effect is augmented in the presence of alcohol.

5. Change in temperature over a wide range has no appreciable effect on the susceptibility of paramecia to alcohol.

6. Aëration and agitation of a *Paramecium* culture renders the paramecia much less susceptible to alcohol.

7. Paramecia in a given solution without food live longer with alcohol than without; starving cultures can even be restored to prosperity by the addition of suitable amounts of any alcohol.

8. Exposure of paramecia to weak ethyl alcohol increases their susceptibility to a stronger dose of ethyl alcohol, and to five other alcohols.

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